APPENDIX 13.2 – A: Bioaerosol Sampling Methods and Limitations

Note: We update the information resource links at least semi-annually, but please let us know if you find a link that has changed by sending an email to **IH_Information_Systems@nehc.mar.med.navy.mil**.

VIABLE AND NON-VIABLE BIOAEROSOL SAMPLING METHODS

Collection Method	Sample Equipment	Analysis Method(s)	Sampling Notes	Information Resource
Impaction into liquid	**AGI-30** (all-glass impinger)	Microscope Culture Bioassay Chemical assay	12.5 LPM d_{50} = 0.31µm; particle collection efficiency ≈ 70% @ 0.5µm; ≈ 90% @ 1µm Sample collection up to 2 hours Liquid can evaporate or spill. Use sterile oil (mineral or glycerol) to prevent evaporation. CANNOT use glycerol for bacterial recovery. Spores can re-aerosolize during collection	www.aceglass.com See Impinger #7540
Impaction into liquid	**BioSampler®** (aka SAC = swirling aerosol collector)	Culture (hardy) Microscope Chemical assay Immunoassay Bioasssay	12.5 LPM Long term collection (up to 8 hrs) with mineral oil Reduced particle bounce Minimal re-aerosolization Liquid can evaporate or spill. Use mineral oil or glycerol to eliminate evaporation (glycerol for PCR & microscope analysis; sterile mineral oil for fungus & bacterial culture). Particle collection efficiency ≈ 85% @ 0.5µm; ≈ 100% @ 1µm	http://www.skcinc.com/prod/Biosampler.asp
Impaction onto stainless or filter	**Andersen Non-Viable 8-stage** (cascade impactor)	Microscope	Aerosol can impact directly onto collection surface or onto filtration media Allows size selection. Cut points at standard collection of 28.3 LPM = 0.4, 0.7, 1.1, 2.1, 3.3, 4.7, 5.8, & 9.0 µm Additional special stages allow 60 & 90 LPM collection for sub-micron particle collection.	http://www.anderseninstruments.com/eight_stage.htm
Impaction onto stainless or filter	**Andersen Personal Cascade Impactor** (personal BZ)		Collect at 2 LPM 8-stage impactor d_{50} stage 1 = 21; stage 2 = 14.8; stage 6 = 1.55; stage 8 = 0.52 µm	http://www.anderseninstruments.com/marple_290_impactor.htm
Impaction onto agar	**Aerotech 6™**	Culture	See Andersen N-6	http://www.aerotechlabs.com/Aero/

VIABLE AND NON-VIABLE BIOAEROSOL SAMPLING METHODS

Collection Method	Sample Equipment	Analysis Method(s)	Sampling Notes	Information Resource
Impaction onto agar	**Andersen** N-6	Culture	Precise speciation; differentiates between *Aspergillus* & *Penicillium* Quantification (CFU/m^3) Collected using high volume pump and agar plates Standard collection is 28.3 LPM for 5 minutes (snapshot sample) Allows size selective sampling based on stages used d_{50} stage 1 = 6.61µm; d_{50} stage 6 = 0.57µm Reports only viable spores (underestimates total spores) 5-20 day incubation Requires sterile collection Routine collection misses species with special growth requirements (e.g., *Stachybotrys*) Requires positive hole correction Requires disinfection between samples to prevent cross-contamination	http://www.anderseninstruments.com/viable_samplers.htm
Impaction onto agar	**Biocassette** (personal BZ)		Single use sampler (disposable)	www.emlab.com/media/resources/biocassette.pdf
Impaction onto agar	**BioStage®-1** Standard BioStage® Micromedia BioStage®	Culture	Replica of the Andersen N-6 bioaerosol sampler. Catalog 225-9535. Single stage; 28.3 LPM; 400 holes; 90-100 mm plates. Catalog 225-9611. Single stage; 14. 15 LPM; 200 holes; 60 mm plates. Catalog 225-9610.	www.skcinc.com http://www.skcinc.com/prod/225-9611.asp
Impaction onto agar	**Burkard Portable Air Sampler**	Culture	Sampling time 1-9 minutes (or continuous) at 10 LPM 90 mm agar plates	http://www.burkard.co.uk/portsamp.htm
Impaction onto agar	**M Air T Tester** (sieve impactor)		Collect at 140-180 LPM; maximum sample volume 1000 L d_{50} = 0.3.5 µm Usually used for clean rooms Sieve collection allows uniform distribution onto agar	http://www.millipore.com/publications.nsf/docs/PF10176EN00
Impaction onto agar	**Mattson-Garvin M/G Air Sampler 220** (slit to agar)	Culture	Agar plate on rotating surface, sampled at 28.3 LPM d_{50} = 0.5 µm Drive motors can be changed to set 1 revolution of the plate at 5, 15, 30, or 60 minutes.	http://www.mattson-garvin.com/models.htm (Mattson-Garvin, Barramundi Corporation)

VIABLE AND NON-VIABLE BIOAEROSOL SAMPLING METHODS

Collection Method	Sample Equipment	Analysis Method(s)	Sampling Notes	Information Resource
Impaction onto agar	**MicroBio MB2**	Culture	Portable Standard sample rate 100 LPM; adjustable sample time Total air volume 25-1000 L Laminar air flow through 1 mm holes onto 55 mm contact agar plate	http://www.aicompanies.com/SBI_products/airsample/MB2/mb2.htm
Impaction onto agar	**RCS** (Reuter Centrifugal Sampler) (collection onto agar strips)	Culture	Standard RCS – Sampling times 0.5, 1, 2, 4, 8 minutes RCS Plus – Typical collection at 50 LPM for 20 minutes; maximum sample volume 2000L. $d_{50} \approx$ 2-5 µm RCS High Flow – Typical collection 100 LPM for 10 minutes; maximum sample volume 1000L. $d_{50} \approx$ 2-5 µm RCS Isolator – Flow rate 100 LPM; maximum volume 1000 L	http://www.biotestusa.com/sampling.html
Impaction onto agar	**SAS** (Surface Air System)	Culture	Collect at 90-180 LPM, using 55, 84, or 90 mm agar plates d_{50} = 0.67µm d_{50} = 1.45µm Requires hole correction SAS Super 100 (100 LPM) can be programmed for collection time and volume	Bioscience International, http://www.biosci-intl.com/products/sas100.htm
Impaction onto agar	**SPS 3000**	Culture	Cascade impaction using 2, 3, or 6 stages. 6 stage hole diameters: stage 1 – 1.20 mm; stage 2 – 0.90 mm; stage 3 – 0.70 mm; stage 4 – 0.50 mm; stage 5 – 0.35 mm; stage 6 – 0.25 mm	http://www.lanzoni.it/ Enter site – Choose 'Enter' over British flag – Choose Products & Services
Impaction onto agar	**STA-203/ 204** (slit to agar collection onto rotating agar surface)	Culture	Sample exposure time (i.e., time for 1 revolution of agar plate) can be adjusted from 2-100 minutes STA 203 - Flow rate 15-50 LPM (30-5000 L total sample volume) STA 204 - Flow rate 15-30 LPM (30-3000 L total sample volume) Includes built-in vacuum pump	www.nbsc.com (New Brunswick Scientific – Choose Biological Air Samplers from the Products list)
Impaction onto agar	**Zefon A6**	Culture	d_{50} = 0.65 µm (1-stage only available)	www.zefon.com

VIABLE AND NON-VIABLE BIOAEROSOL SAMPLING METHODS

Collection Method	Sample Equipment	Analysis Method(s)	Sampling Notes	Information Resource
Impaction onto tape or slide	**Air-O-Cell™ cassette** (sticky sampling media)	Microscope	Quick, easy to use Collect viable & non-viable particles using high volume pump & Air-O-Cell cassette (37 mm) Standard sampling is 15 LPM for 10 minutes; flow rate range 5-30 LPM d_{50} = 2.6 µm @ 15 LPM Particle collection efficiency @ 15 LPM ≈ 0 @ 2µm; 85% @ 3µm; 95% @ 15-80µm Can get identification & quantification (spores/m^3 & speciation) Cassettes average $7-$10 each; long shelf life Specially adapted pump available from Zefon (MiniPump) & from SKC (QuickTake 15)	www.zefon.com http://www.skcinc.com/prod/225-9501.asp
Impaction onto tape/ slide	**Burkard Continuous Recording Personal Air Sampler**	Microscope	Personal sampling continuously over 24 hour period Particle collection efficiency ≈ 90% for particles ≥ 5µm Preset selection for 2 mm/hr for 24 hr; 4 mm/hr for 12 hrs; or 8 mm/hr for 6 hr Allows timed sample collection over 24-hr intervals	http://www.burkard.co.uk/contas.htm
Impaction onto tape/ slide	**Burkard Spore Trap** (personal sampler on moving slide)	Microscope	d_{50} = 2.52 µm; very poor collection efficiency of particles <2 µm Poor particle deposition uniformity Collect at 10 LPM for 1-9 minutes	http://www.burkard.co.uk/perssamp.htm
Impaction onto tape/ slide	**Burkard Spore Trap – 7 day Recording Sampler** (rotating drum)	Microscope	Tape/slide on rotating drum, allowing 1-7 days continuous recording 10 LPM - drum rotates 1 revolution/7 days @ 2mm/hour. Allows timed sample collection to follow fluctuations over 24-hr interval. d_{50} = 5.2 µm; Can improve collection efficiency of particles <10 µm by changing orifice. Rugged equipment. Usually used for outdoor sampling, especially air quality testing, e.g., pollen counts.	http://www.burkard.co.uk/7dayst.htm
Impaction onto tape/ slide	**Cyclex™** (circular bioaerosol impact sampler)	Microscope	d_{50} = 1.8 µm Collect at 20 LPM for up to 10 minutes Collects non-viable samples using aluminum 360° impaction chamber.. Can also be used for collecting carpet and cavity samples with accessories.	http://www.emssales.net

VIABLE AND NON-VIABLE BIOAEROSOL SAMPLING METHODS

Collection Method	Sample Equipment	Analysis Method(s)	Sampling Notes	Information Resource
Impaction onto tape/ slide	**Cyclex-D™ cassette**	Microscope	Bioaerosol sampling for fungal spores, pollen, fibers, dander, and others. Used with a high volume pump; 20 LPM for 1-15 minutes Impacts onto transparent adhesive slide in the center of the cassette. Particle collection efficiency ≈ 65% @ 1µm; 82% @ 2 µm; 98% @ 3 µm $d_{50} \approx 1\mu m$	http://www.emssales.net
Impaction onto tape/ slide	**Micro5 Microcell** (personal sampler)	Microscope	Personal and area sampling; 5 LPM for 1-15 minutes $d_{50} \approx 1\mu m$ Particle collection efficiency ≈89% @ 1µm;	http://www.emssales.net
Impaction onto tape/ slide	**MK-3** (moving slide)	Microscope	Sample collected on greased slide at 15 LPM $d_{50} = 2.0\ \mu m$	Purchase http://www.emssales.net/productlist.aspx?CategoryID=2&SubID=7 Information http://www.gapenviromic.com/images/MK_3_Air_Sampler_Operation.pdf
Impaction onto tape/ slide	**Rotorod** (tape on rotating rod)	Microscope	Typically used for air quality monitoring (pollen counts)	http://www.multidata.com/ProductCatalog.html
Impaction onto tape/ slide	**VPPS 1000** (volumetric particle sampler using rotating drum)	Microscope	10 LPM for 24-hour sampling period (slide moves at 2 mm/hr) Can be set for 1-7 days continuous recording	www.lanzoni.it Enter site – Choose 'Enter' over British flag – Choose Products & Services
Impaction onto tape or slide	**WallChek™** **(w**all cavity sampling)	Microscope	Provides essentially non-destructive sampling in wall cavities (requires a 3/8" hole). A special adaptation using an Air-O-Cell cassette.	www.aerotechlabs.com/Aero http://www.skcinc.info/instructions/37504.pdf

VIABLE AND NON-VIABLE BIOAEROSOL SAMPLING METHODS

Collection Method	Sample Equipment	Analysis Method(s)	Sampling Notes	Information Resource
Filtration	**Button™ Aerosol Sampler** (inhalable mass)	Culture Microscope	25 mm filter; 4 LPM sample collection; Collects inhalable fraction Reduces electrostatic effects Good filter collection uniformity Particle collection efficiency ≈100% for 0.5 – 5 µm particles when sampling long term (some sensitive species may dessicate). Good for collecting bacteria if using gelatin filter	http://www.skcinc.com/prod/225-360.asp
Filtration	**Cassette** 25 mm 37 mm 45 mm	Microscope Culture (hardy species) Bioassay Chemical assay Immunoassay	Usually collected at 1-5 LPM Particle collection efficiency good at 0.5µm and above Sterile filters required for culturing Filters with grids can be purchased for counting Electrostatic charges may be collection problem Filtration methods tend to concentrate particles at center of filter Good collection method for long-term sampling of hardy spores	www.zefon.com http://www.skcinc.com/prod/gelfilters.asp
Filtration	**IOM Sampler**	Culture Microscope	Use sterilized 25 mm polycarbonate filters to collect personal bioaerosol samples. Use multidust foam discs to size select into inhalable, thoracic, and respirable fractions. Flow rate 2 LPM	http://www.skcinc.com/prod/225-70A.asp
Filtration	**Laro-100**	Culture Microscope	Uses 0.8 µm MCEF filters. Claims to capture 100% of particles from 1-10 µm. 4-5 LPM for 3-30 minutes. Can be used for 8-hr personal sample collection at 1-2 LPM flow rate.	http://www.laro100.com./ http://www.emsl.com/laro_sampling_guide.html

SURFACE AND BULK SAMPLING METHODS

Collection Method	Sample Equipment	Analysis Method(s)	Sampling Notes	Information Resource
Bulk	Bulk material or dust	Cultured or direct microscope exam CFU/g	Carpet or dust sampling 25 mm (with conductive cowl) or 37 mm cassettes with beveled inlet port	Carpet sampling pump kit - http://www.skcinc.com/prod/225-9541.asp MicroVac - www.zefon.com
Swab	Bulk material or dust with sterile swab (Q-tip or cotton ball will work)	Direct microscope or culture CFU/m^2	Quick Inexpensive Measured template required for quantification Surfaces may not adequately characterize extent or type of contamination No correclation to airborne results Swab & template kits available	www.zefon.com http://www.skcinc.com/prod/sterilswab.asp
Tape	Clear sticky tape placed directly over the surface to be sampled. Place tape onto slide for transport	Microscope	Quick Inexpensive Kits available, e.g., BioTape™ (flexible plastic slide with adhesive center from Zefon),	Zefon http://www.zefon.com

OTHER MICROBIOLOGICAL COLLECTION METHODS

Resource Information Available from Laboratories Offering These Tests (See Laboratory Services Chart, Appendix C)

Analyte	Sample Information	Analysis Method(s)	Sampling Notes
Allergens	Impinger methods Filter collection followed by Wash Settled dust	Immunoassay (IA) (antigen-antibody specific)	Analyzes viable & non-viable for available antigenic species. Quick analysis. Can be fluorescent IA, enzyme IA [ELISA], or radio IA [RAST] Special equipment & expertise needed. Cross-reactivity can occur. Typical sample allergens = dog, cat cockroach, dust mites
β glucan (1→3)-β-D glucan (fungal cell wall component)	Filtration: 0.8µm cellulose acetate/ nitrate filter or 1 µm polycarbonate	Chemical assay or Bioassay (LAL)	β glucan is a cell wall component of all fungi; sampling can be used as biomarker for the presence of any fungal contamination Detects dead & living spores Does not identify which fungal species is present Quick analysis time
Endotoxins (Gram negative bacteria)	Filtration: 37 mm cassette with polycarbonate filter Can use bulk sample	Bioassay (LAL) Chemical assay via GC-MS or HPLC Kinetic chromogenic assay	Cassettes/filters/vials must be sterile and endotoxin-free Identifies toxins produced by gram negative bacteria Can analyze air, dust, or bulk material samples (including water samples). May be useful as a post-remediation sample after a sewage spill or backup. Have shown correlation between occupant symptoms & endotoxin concentrations
EPS (bacteria & fungi)		Immunoassay	EPS = Extracellular polysaccharides
Ergosterol (fungal cell wall component)	Filtration 0.45µm polycarbonate filter (closed face) 1-4 LPM	Chemical assay (HPLC, MS or GC-MS)	Ergosterol is the main membrane sterol in most fungi; sampling is used as biomarker for the presence of fungal contamination Detects dead & living spores Long-term stability before analysis Does not identify which fungal species is present Reported in µg ergosterol
Fatty Acids (bacteria)		Chemical assay	Fatty acids are cell wall components of bacteria. Analysis uses gas chromatography to determine the fatty acid profile, then compares results to a reference database using statistical pattern recognition software.
MVOCs (Microbial Volatile Organic Compounds)	Low flow pump with sorbent tube (e.g., Anasorb 747 carbon) 0.2 LPM for 240 minutes	Chemical assay (GC-MS)	Mold must be actively growing for organic compound production. Samples must be frozen after collection. Samples can be collected using summa canisters. Little consensus on which MVOCs are “important” or medically significant.
Mycotoxins (Fungi)	Filtration: 37 mm cassette With 1 µm PTFE (#003-2028-00)	Chemical assay (HPLC; GC/MS; LC/MS)	Sensitive analytical methods not available for most indoor air mycotoxins. Agriculturally important toxins methods widely available. Identifies secondary metabolites of fungi, produced only when the fungus is stressed.

OTHER MICROBIOLOGICAL COLLECTION METHODS **Resource Information Available from Laboratories Offering These Tests (See Laboratory Services Chart, Appendix C)**			
Analyte	**Sample Information**	**Analysis Method(s)**	**Sampling Notes**
PCR Genetic Identification	Filter: 37 mm cassettes with 1 µm PTFE filter	Bioassay, i.e., PCR (polymerase chain reaction)	Genetic identification using genus or species-specific DNA primers Analysis within 1 day Expensive; requires expertise & special equipment DNA primers limited (mostly *Aspergillus, Penicillium, Stachybotrys*)

Abbreviations Key:

β	beta	GC	gas chromatography	LAL	*Limulus* amoebocyte lysate assay
BZ	breathing zone	MS	mass spectrometry	PTFE	polytetrafluoroethylene
m^3	cubic meters	HPLC	high performance liquid chromatography	PCR	polymerase chain reaction
≈	approximately	mm	millimeters	LC	liquid chromotography
µm	micrometers				
LPM	liters per minute				
MVOC	microbial volatile organic compounds				
d_{50}	cut-off diameter or cut size**;** the aerodynamic particle size at which 50% of the particles will be captured by the collection method of interest.				

Analysis Methods:
Culture (viable) – collect or transfer sample onto nutrient agar to grow colonies
Microscope (non-viable) – examine under microscope for counting and/or morphological identification
Bioassay – analytical method in which the result is an observable effect on or in a biological system/ organism. Includes genetic identification using DNA primers (i.e., polymerase chain reaction (PCR)) and LAL assay for endotoxins.
Chemical Assay – analysis of chemical compounds produced by or contained in the microbe, i.e., MVOCs, mycotoxins
Immunoassay – analysis based on the specificity of an antigen-antibody reaction (e.g., cat, dog, cockroach, & dust mite allergen tests; ELISA)

	Advantages	Disadvantages
Culture:	Species identification	Slow results (7-10 days incubation)
	Large reference database	Media is selective
	No special equipment needed	Underestimates total count
		Detects only living organisms that are able to live & compete on selected nutrient media & conditions
		Species cultured from a site may not be the most prevalent or important
Microscope:	Analyzes total count	Limited identification ability; cannot distinguish some species
	Fast	Background debris can interfere with identification
	Affordable	Special expertise needed for confident identification
Biological & Chemical Assay	Fast	Special equipment & expertise needed
	Analyzes viable & non-viable	Limited reference data except for endotoxins
	Can screen for specific genus/species	